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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002950713 for a patent by SIMON MICHAEL WEST and DAVID KANNAR as filed on 09 August 2002.

I further certify that the above application is now proceeding in the name of VITAL HEALTH SCIENCES PTY LTD pursuant to the provisions of Section 113 of the Patents Act 1990.

WITNESS my hand this
Fifteenth day of August 2003

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT AND SALES

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Patents Act 1990

PROVISIONAL SPECIFICATION

Invention title: Carrier

The invention is described in the following statement:

Carrier

Field of the Invention

This invention relates to a carrier for use in the topical administration of pharmaceutical or pharmacologically active compounds.

Background of the Invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not to be taken as an admission that the document, act or item of knowledge was at the priority date:

(a) part of common general knowledge; or

(b) known to be relevant to an attempt to solve any problem with which this specification is concerned.

The major objective in topical drug delivery is to obtain an appropriate biological effect at a desired site of action. The choice of carrier used in the administration of pharmaceutical or pharmacologically active compound can be critical to the efficacy of the topically delivered pharmaceutical or pharmacologically active compound. Whether the pharmaceutical or pharmacologically active compound is to be used for localised topical delivery or transdermal delivery, absorption mechanisms are assumed to be similar. Inherent biological activity of a pharmaceutical will however, be irrelevant if it does not possess the correct physiochemical properties to allow release from the formulation of the biologically active form in the target site of action after passage across the skin. This has been found to be somewhat dependant upon the following characteristics.

Drug Transfer through the skin

When a drug is released from a formulation it will first partition into the outer lipids of the stratum corneum. The degree of absorption will depend primarily upon solubility of the drug into these lipids and partition co-efficient of the drug between the skin and release from the formulation. A simple method for maximizing this is to choose formulation components that allow the drug dose to reach its solubility limit. Ostrenga et al. demonstrated this principle by improving solubility and partition characteristics of two corticosteroids through manipulating the formulation ratio of water and propylene glycol. In this study the most effective formulations were those that contained adequate propylene glycol to solubilise the maximum drug concentration in the finished pharmaceutical product.

It is also reported that supersaturated systems provide thermodynamic activity greater than unity that enhances skin penetration of drugs. A drug solvent system using a mixture of volatile and non-volatile solvents as vehicles, where the volatile compounds evaporate from the skin, can create a supersaturated solution on the skin surface and stimulate drug absorption. It is thought that some transdermal patch delivery systems have the ability to absorb water from the skin increasing thermodynamic activity of a drug creating a supersaturated solution thereby promoting its passage through the skin. One of the major problems with use of mixed volatile and non volatile delivery systems however, is the difficulty in creating systems that are reproducible, as the rate and degree of volatile evaporation will depend, to a large extent upon ambient conditions during application. Variability in absorption kinetics causes fluctuations in drug delivered and unreliable clinical efficacy.

When a suitable solvent system cannot be identified suspensions may be used. In these formulations, particle size of the incorporated drug compound can significantly influence effective absorption. This was demonstrated by Barrett et al. using a variety of fluocinolone acetonide formulations with different particle size. The formulations were applied to forearm skin of volunteers and degree of vasoconstriction measured. The effect was greatest in those formulations using micronised drug that had been taken into solution with propylene glycol. It was concluded that solubility and partition characteristics of a drug were clearly important parameters in formulating to promote skin absorption. In theory this means that drugs with good oil and water solubility and balanced partition coefficient, will better penetrate the skin.

Modern drugs typically do not have optimal solubility characteristics, and this is currently quantified by use of a solubility parameter. This has been estimated to be approximately 10 for the skin, so drugs with solubility parameters similar to this may be expected to be freely soluble creating a large concentration gradient across the skin or high partition co-efficient. The importance of this is evident in an analysis of skin permeability data by Potts and Guy who examined the permeability of 90 compounds in aqueous solution and determined that permeability coefficient (K_p) through the skin was related to their octanol-water partition coefficient and the molecular weight in the following relationship:

$$\text{Log } K_p (\text{cm s}^{-1}) = -6.3 + 0.71 \log P - 0.0061 \text{ MW} \quad (r^2 = 0.69)$$

This emphasizes the importance of solubility and partition coefficient, but like many mathematical structure activity relationships, results in a 2 dimensional answer to a three dimensional problem. For example, flux through the skin using this equation results in a

parabolic dependency on the partition coefficient which is still unclear. If a true linear concentration gradient existed then the higher the concentration gradient, the higher the drug absorption. The fact that the relationship is not linear suggests that physical limits exist, such as the number of pores in the skin or physiochemical forces other than solubility, dissolution and dispersion also act to facilitate membrane transport. It has been suggested that at high log P (a highly lipophilic compound), the transfer rate out of the stratum corneum is rate limiting or that drugs with high log P values generally have poor aqueous solubility. This means that lipid soluble drugs tend to stay in the phospholipid membrane because by nature they are lipophilic, that is, the drugs are trapped in the skin and not released into the target site.

Based on the equation and the accompanying assumption that drugs are transported across skin by virtue of a concentration gradient, it is suggested that drugs with log P in the range of 1 to 3 are more likely to diffuse through the skin. However, this simply serves to identify drugs that may move easily through the skin. This does not help to improve the transport of poorly soluble, highly lipophilic drugs.

Skin Enhancers

Many modern drugs are highly lipophilic skin enhancers and various formulation techniques have been developed to improve their absorption through the skin. Skin enhancers typically function to modify structure especially of the stratum corneum by dissolving or interfering with the lipid matrix to improve permeability of drug compounds. Examples include compounds like capric acid, oleic acid, azone, decylmethyl sulfoxide and hydroxy cinnamates. Dermal absorption of progesterone for example increases by 143% when the stratum corneum is delipidized. The enhancement increases to 843% when the stratum corneum is totally eliminated. With such aggressive modification, commonly reported problems with repeated use of such systems are obvious to those trained in the art and include contact dermatitis, reddening of the skin, itching and burning that requires movement of the patch or application of the drug, around the body to prevent local irritation. The reddening is said to disappear within hours of removing the patch. But concern has been raised with respect to long term risk and safety of use of this type of transdermal delivery system, mainly because increased drug permeability is achieved at the cost of damaging a fundamentally important protective layer of the skin.

A study by Morgan, TM., Parr, RA., Reed, BL. and Finnin, BC (1998). Enhanced transdei delivery of sex hormones in swine with a novel topical aerosol. *J. Pharm. Sci.* 87(10): 12 1225 investigated the transdermal delivery of testosterone and estradiol in pigs using a n metered dose topical aerosol containing a penetration enhancer padimate O. Metered dc devices require co-ordination and manual dexterity for efficient use. The authors also cla that the dose system provides flexibility and can be moved around to provide a greater surface area of application. Further, it would still be necessary to move the dose around because the enhancer is an hydroxy cinnamate that damages the skin and causes irritati and erythema. Therefore, this aerosol dosing system would offer no more advantage tha patch containing enhancers.

A suitable carrier capable of delivering a broad range of pharmaceuticals pharmacologically active compounds and improving absorption of the pharmaceutic pharmacologically active compound in the targeted area without damaging the sk therefore required.

Summary of the Invention

It has surprisingly been found that a carrier composition comprising complexes of tocoph phosphate mixed with pharmaceuticals or their phosphorylated analogue allows rapid an efficient transport of the pharmaceuticals or pharmacologically active compounds throug skin with no evidence of inflammation or disruption. This carrier can be used for therapie that require chronic administration and where the carrier needs to have reduced side effe and improve the well being of the patient.

According to the first aspect of the invention, there is provided a carrier composition for u topical administration of pharmaceuticals and pharmacologically active compounds, said carrier comprising an effective amount of one or more complexes of tocopheryl phosphat

Preferably, the amount of the one or more complexes of tocopheryl phosphate is in the ra of from 0.1 to 5 % (w/w).

According to a second aspect of the invention, there is provided a method for improving t efficacy of topically administered pharmaceuticals and pharmacologically active compour said method comprising the step of incorporating the pharmaceutical or pharmacologicall active compound in a carrier comprising an effective amount of one or more complexes o tocopheryl phosphate.

The present invention also provides use of an effective amount of one or more complexes of tocopheryl phosphate together with other excipients in the manufacture of a carrier for use in the topical administration of pharmaceuticals or pharmacologically active compounds.

The present invention also provides a carrier when used in the topical administration of pharmaceuticals or pharmacologically active compounds, the carrier comprising an effective amount of one or more complexes of tocopheryl phosphate.

The present invention also provides a pharmaceutical composition comprising one or more pharmaceuticals or pharmacologically active compounds and a carrier comprising an effective amount of one or more complexes of tocopheryl phosphate.

The term "complexes of tocopheryl phosphate" refers to the reaction product of one or more phosphate derivatives of tocopherol and one or more complexing agents selected from the group consisting of amphoteric surfactants, cationic surfactants, amino acids having nitrogen functional groups and proteins rich in these amino acids as disclosed in international patent application no PCT/AU01/01476.

The preferred complexing agents are selected from the group consisting of arginine, lysine and tertiary substituted amines, such as those according to the following formula:

$$NR^1R^2R^3$$

wherein R^1 is chosen from the group comprising straight or branched chain mixed alkyl radicals from C6 to C22 and carbonyl derivatives thereof;

R^2 and R^3 are chosen independently from the group comprising H, CH_2COOX, $CH_2CHOHCH_2SO_3X$, $CH_2CHOHCH_2OPO_3X$, CH_2CH_2COOX, CH_2COOX, $CH_2CH_2CHOHCH_2SO_3X$ or $CH_2CH_2CHOHCH_2OPO_3X$ and X is H, Na, K or alkanolamine provided R^2 and R^3 are not both H; and

wherein when R^1 is RCO then R^2 may be CH_3 and R^3 may be $(CH_2CH_2)N(C_2H_4OH)$-H_2CHOPO_3 or R^2 and R^3 together may be $N(CH_2)_2N(C_2H_4OH)CH_2COO$-.

Preferably, the complex of tocopheryl phosphate is laurylaminodipropionic acid tocopheryl phosphate.

Preferably, the tocopheryl phosphate from which the complex is prepared consists of a mixture of one mono-tocopheryl phosphate derivative and one di-tocopheryl phosphate derivative wherein the amount of mono-tocopheryl phosphate derivative is no less than

equimolar to the amount of di-tocopheryl phosphate derivative as disclosed in international patent application no PCT/AU01/01475. For example, a mixture containing 70% tocopheryl phosphate and 26% di-tocopheryl phosphate.

The term "pharmaceutical or pharmacologically active compound" is used herein to refer to pharmaceutically active compounds for human or veterinary application. Examples of pharmaceutical compounds include but are not limited to narcotic analgesics such as morphine and levorphanol, non narcotic analgesics such as codeine and acetaminophen, corticosteroids such as cortisone, anaesthetics such as propofol, antiemetics such scopolamine, sympathomimetic drugs such as adrenaline and dopamine, antiepileptic drugs such as fosphenytoin, anti-inflammatory drugs such as ibuprofen, thyroid hormones and antithyroid drugs including thyroxine, phytochemicals including α-bisabolol, eugenol, silybin, soy isoflavones, iridoid gylcosides including aucubin and catalpol, sesquiterpene lactones including pseudoguaianolide from Arnica chamissonis, terpenes including rosmarinic acid and rosmanol, phenolic glycosides including the salicylates salicin, saligenin and salicyclic acid, triterpenes taxasterol or α-lactucerol, and isolactucerol, *p*-hydroxyphenylacetic acid derivative taraxacoside, hydroquinone derivatives including arbutin, phenylalkanones including gingerols and shagaols, hypercin, and acylphloroglucides including xanthohumol, lupulone, humulone and 2-methylbut-3-en-2-ol. The pharmaceutical or pharmacologically active compound can be in any suitable form including phosphate derivatives.

A person skilled in the art would know which other excipients could be included in the carrier. The choice of other excipients would depend on the characteristics of the pharmaceutical or pharmacologically active compound. Examples of other excipients include solvents, surfactants, emollients, preservatives, colorants, fragrances and the like. The choice of other excipients will also depend on the form of topical administration used. The form of topical administration used may be any suitable delivery systems considered by those skilled in the art as capable of delivering drugs topically on human or other animal skin to achieve a systemic or dermal effect. It includes but is not limited to creams, lotions, gels, emulsions, liposomes, aerosols, patches, poultices, subcutaneous depots, plasters and sustained release systems designed to alter absorption kinetics in favor of zero order release.

Brief Description of the Drawings

Figure 1: Changes in total estrogens (mean ± SE) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 0.17µg of estrogen (E) or estrogen phosphate (EP) were applied.

Figure 2: Changes in total estrogens (mean ± SE) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 0.17µg of E or EP in ethanol were applied.

Figure 3: Percent change in tritiated E vs tritiated EP in ovariectomised hairless rats to which formulations containing tritiated E or EP were applied.

Figure 4: Changes in total testosterone (mean ± SD) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 1.00 µg ± 0.02 µg of T or TP were applied.

Examples

The invention is further explained and illustrated by the following non-limiting examples.

Example 1

A carrier cream according to the invention was prepared as follows:

PHASE A	W/W
Deionized water	61.95%
Glycerin	5.00
Trisodium EDTA	0.05
Carbomer (Carbopol Ultrez 10)[2]	0.50
laurylaminodipropionic acid tocopheryl phosphate[1]	7.50
PHASE B	
Cetearyl Alcohol (and) Ceteareth-20 (Phoenoxol T)[3]	2.00
Glyceryl Stearate (Emerest 2400)[4]	1.00
Isopropyl Myristate (Pelemol IPM)[3]	5.00
Cetyl Ethylhexanoate (Pelemol 168)[3]	3.50
Isocetyl Behenate (Pelemol ICB)[3]	3.50
Oleyl Erucate (Cetiol J-600)[4]	3.00
Dimethicone (Dow 200,100 cSt.)[5]	0.50
PHASE C	
Deionized Water	5.00
Triethanolamine (99%)	0.50
PHASE D	
Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben (Germaben II)[6]	1.00

1. Vital PC, Incorporated
2. B.F. Goodrich, Incorporated
3. Phoenix Chemical, Incorporated
4. Cognis, Incorporated
5. Dow-Corning, Incorporated
6. ISP Corporation

Procedure:

Procedure: Combine Phase A items minus the Carbomer and laurylaminodipropionic acid tocopheryl phosphate with stirring. When a solution is obtained, disperse Carbomer in this solution. Begin heating Phase A to 70-75° C. with adequate agitation. Disperse laurylaminodipropionic acid tocopheryl phosphate in Carbomer mucilage with sweep agitation. Combine Phase B items and heat to 75-80° C. with adequate agitation. With Phase A uniform and at 70-75° C. and Phase B uniform and at 75-80° C. Add Phase B to Phase A with adequate agitation. Allow AB to cool to 50° C. and then add Phase C solution to AB. Continue adequate agitation of ABC until 45° C. is reached. Add Phase D to ABC. Continue adequate agitation until 35° C is reached.

Example 2

The transdermal delivery of estradiol and estradiol 3:phosphate in the hairless rat model was evaluated in this example.

Methods

Animals: 23 female albino hairless rats were ovariectomised under isoflurane-induced anaesthesia and allowed to recover for 10 days prior to experimentation. This should allow clearance of any estrogens from the body.

Blood sampling: Blood samples (500 µl) were obtained from the tail vein of conscious restrained rats at 0, 1, 2, 4, 8, 16 and 24 hours following application of both the estradiol (n=5) and estradiol phosphate (n=6) formulations. Blood was collected into EDTA tubes, then centrifuged at 5000 rpm for 10 minutes. Plasma was removed and stored at –80°C until assayed.

Transdermal Formulation Preparation and Application: estradiol and estradiol phosphate were provided by Tocovite Pty Ltd and prepared at concentrations of 20 µg/ml approximately 1 hour before application in the carrier cream from Example 1.

Estradiol Phosphate (***EP***): 4.3 mg of EP was dissolved in 17.3 ml of acetone (0.25 mg/ml). 20 µl was transferred to an Eppendorf tube and the solvent was evaporated in a nitrogen stream. Then 0.999 g of the carrier cream from example 1 was added, and mixed with a glass rod and centrifuged. This was repeated 5 times. Final concentration = 4.90 µg/ml.

Estradiol (***E***): 6.7 mg was dissolved in 26.8 ml of absolute ethanol (0.25 mg/ml). 20 µl was transferred to an Eppendorf tube and the solvent was evaporated in a nitrogen stream. Then

1.003 g of the carrier cream from example 1 was added, and mixed with a glass rod and centrifuged. This was repeated 5 times. Final concentration = 4.89 µg/ml.

E and EP formulations in ethanol: 0.5 mg of E and EP was mixed with 50 ml portions of ethanol. 20 µl of these solutions was directly applied to the skin.

Each formulation was applied to the dorsal skin of an anaesthetised rat in an area of approximately 4 cm^2 marked with an indelible felt tip marker. Application of approximately 30 ± 3.2 mg of formulation (containing 0.15 ± 0.02 µg of E or EP) was applied to the site with a curved glass rod applicator. The formulation was 'rubbed' in until it appeared to have been absorbed into the skin, which took between 5-10 min. Any changes in the consistency of the formulation during this procedure were noted The amount of formulation applied and the area of the application site were weighed for each animal.

Organ Collection: After 24hr monitoring animals were killed with an overdose of anaesthetic. All organs were removed, weighed and stored at –80°C until assay.

Total Estrogens RIA: The RIAs were performed using a commercially available total estrogens kit (ICN Pharmaceuticals, catalogue # 07-140205) with 100 % cross-reactivities for 17β-estradiol and estrone. The standard curve range for this assay is 2.5 – 100 pg/ml (r^2 - 0.943). Extraction efficiency was determined through a series of spiking assays and was between 90 to 98% using diethyl ether as the extraction solvent for rat plasma and organs. This solvent did not interfere with the assay. Plasma volumes of 100 µl were used for assay.

Results

Formulation Application: The average areas (± SE) to which formulations were applied on the dorsum of the animals were 3.88 ± 0.03 cm^2 and 3.88 ± 0.07 cm^2 for the E and EP groups respectively. The average amounts of these formulations applied in the E and EP groups were 0.15 ± 0.02 µg. No symptoms of inflammation were observed in the study such as erythema or eodema.

Total Estrogens in Plasma: Measurable levels of estrogens (between the standard curve range of 2.5 – 100 pg/ml) were present in both groups of animals with maximum concentrations of 16.63 ± 8.18 (mean ± SE) pg/ml plasma measured in the E group at 2, 8 and 16 hr post-application and a maximum concentration of 49.16 ± 13.21 pg/ml plasma measured at 16 hr post-application in the EP group (Figure 1). Baseline measurements taken at t=0 were subtracted from all values to correct for background levels present in the plasma.

Figure 1: Changes in total estrogens (mean ± SE) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 0.17µg of E or EP in the carrier from Example 1 were applied.

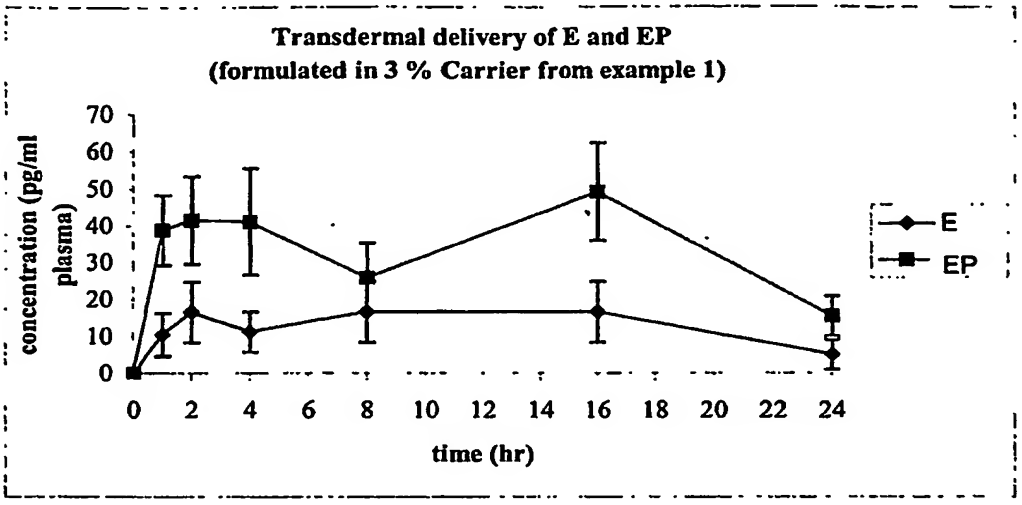
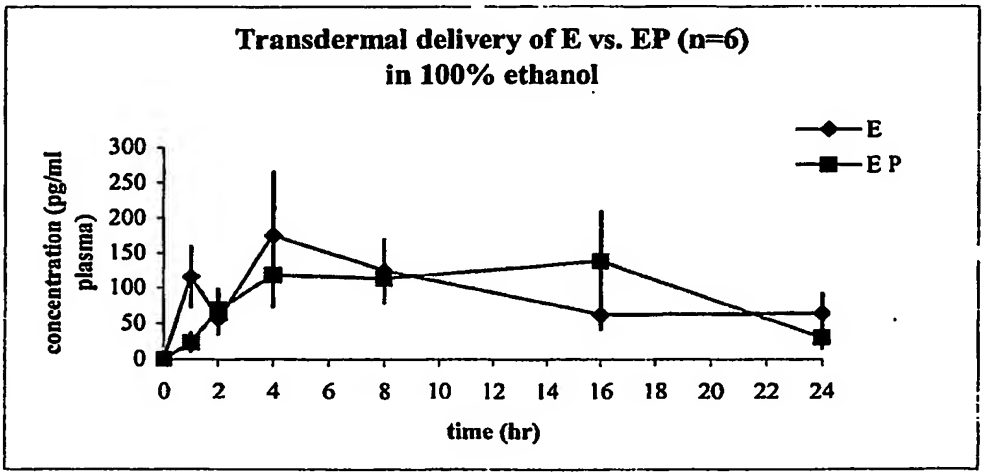


Figure 2: Changes in total estrogens (mean ± SE) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 0.17µg of E or EP in ethanol were applied.



Discussion

This study evaluated the transdermal delivery of EP and E in female hairless rats. The concentration of estradiol in blood was consistently higher when estradiol phosphate was

applied over a 24-hour period (statistically significant, *P<0.01* at 2, 4 and 16 hours). At the equivalent doses that were applied the EP resulted in at least twice the plasma concentration of the hormone compared to the E treatment. This clearly demonstrates that EP delivered in the carrier from example 1 may provide a more effective formulation for delivering E. Interestingly the amount of free estradiol delivered after application of E in the carrier from example 1 was also quite significant. Most importantly, neither the E or EP treatment produced any inflammatory symptoms.

Morgan et al. (Morgan TM, O'Sullivan HMM, Reed BL, Finnin BC. Transdermal Delivery of Estradiol in Postmenopausal Women with a Novel Topical Aerosol. *J. Pharm. Sci.* 1998;87(10):1226-1128) delivered 3 mg of estradiol daily a carrier containing the skin enhancer padimate O containing over 30 cm^2 in 4 post menopausal women for 9 days. At the end of the study period mean blood levels of estradiol 24 hours post dose were 53 ± 7 pg/ml measured with commercial radio immunoassay kits measuring both estradiol and estrone. This was said a significant 4 fold improvement from a baseline level of circulating estradiol of 13 ± 5 pg/ml and deemed to be a clinically relevant dose. In contrast, in this example maximum plasma concentrations of 16.63 ± 8.18 (mean ± SE) pg/ml were detected in the E group at 16 hr post-application and a maximum concentration of 49.16 ± 13.21 pg/ml at the same time point in the EP group (Figure 1) following 0.17 µg applied over an average surface area on the dorsum of the animals of 3.88 ± 0.03 cm^2 and 3.88 ± 0.07 cm^2 for the E and EP groups respectively. Ignoring the differences in skin physiology between the animal model and human skin, approximately the same estradiol levels were achieved even though substantially smaller doses were used in the compositions containing a carrier according to the invention.

Although not completely valid because different models were used, comparison with other studies reveal that the carrier according to the invention stimulated transport of estradiol through the skin.

Conclusion

The trial demonstrated that useful doses of estradiol may be delivered based on the hairless rat model and it may be inferred from the similarity of the properties of the hairless rat to human skin, that estradiol phosphate formulated in the manner proposed in this invention, may prove to be efficacious for hormone replacement therapy. Interestingly the extremely low drug doses utilised in this example managed to deliver potentially therapeutic doses of

estradiol. It is quite clear that the carrier was able to release significant amounts of free estradiol into the blood and is therefore likely to promote the required biological response at the site of action.

The trial also demonstrated that the carrier utilised in both treatment arms not only improved the absorption of estradiol phosphate but of estradiol. This suggests that carrier dependant stimulation of absorption was independent of the drug analogue used.

Without wishing to be bound by theory, the significant improvement of transport appears to be due to the benign interaction of the carrier according to the invention with the lipids in the stratum corneum and may be related to the unique surfaction system of the carrier of this invention.

Consistent with previously published literature on ethanol formulations, greater amounts of estradiol are delivered through the skin which is probably due to cellular disruption caused by the stripping of the stratum corneum. However, following application of the ethanol formulation, skin irritation, erythema and damage was observed. There was no irritation of the skin when the carrier from Example 1 was used.

Example 3

The acute transdermal penetration of ^{3}H-Estradiol (E) and ^{3}H-Estradiol Phosphate (EP) in the hairless rat model was evaluated in this example.

Methods

Animals: 6 female albino hairless rats were used in this study (n=3 per treatment group).

Transdermal Formulation Preparation and Application: ^{3}H-E and ^{3}H-EP were provided by Tocovite Pty Ltd and prepared in formula approximately 1 hr before application in in the carrier cream from Example 1.

20 µl of ^{3}H-E and ^{3}H-EP were aliquoted into 1 ml Eppendorf tubes. The solvents from both ^{3}H-E and ^{3}H-EP were evaporated under a stream of nitrogen. Once completely dry 0.498 g of the carrier cream of example 1 was added to ^{3}H-E and 0.502 g to ^{3}H-EP and mixed with a glass rod and centrifuged for 1 minute. This was repeated 5 times.

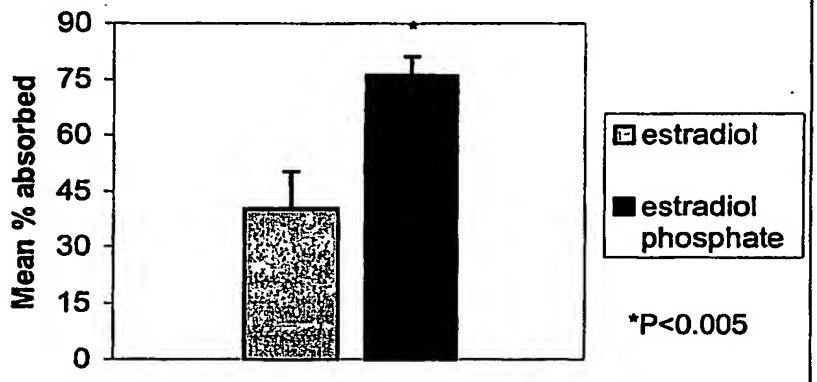
Each formulation was applied to the dorsal skin of an anaesthetised rat in an area of approximately 4 cm^2 marked with an indelible felt tip marker. Application of approximately 30 mg of formulation (containing 5 µg of ^{3}H-E and ^{3}H-EP) was applied to the site with a curved

glass rod applicator. The formulation was 'rubbed' in until it appeared to have been absorbed into the skin, which took between 5 to 10 minutes. A tegaderm (3M) patch was applied to the area to prevent animals from removing the formulation.

Results and Discussion

This study clearly demonstrates that EP was more readily absorbed in comparison to E when transdermally applied using the invention (Figure 3). While the drug form had a significant impact on improving the amount of estradiol delivered it is important to note that the carrier stimulated rapid transport of both drug analogues through the skin. Analysis of individual skin layers was also undertaken in this study and revealed that minimal E or EP remained in the skin 24 hours after application. Higher levels of EP were found in the epidermis and dermis due to higher volumes of the EP moving through the skin during the 24-hour period.

Figure 3: % Absorption of tritiated E vs EP in hairless rat model after 24 hr dermal application



Conclusion

The trial demonstrated that useful doses of estradiol may be delivered based on the hairless rat model. It is quite clear that the carrier was able to release significant amounts of free estradiol into the blood and is therefore likely to promote the required biological response at the site of action.

The trial also demonstrated that the carrier utilised in both treatment arms not only improved the absorption of estradiol phosphate but of estradiol. This suggests that carrier dependant stimulation of absorption was independent of the drug analogue used.

Example 4

The transdermal delivery of testosterone and testosterone phosphate in the hairless rat model using the carrier from Example 1 was investigated in this example.

Methods

Animals: 12 Female albino hairless rats were ovariectomised under isofurane-induced anaesthesia and allowed to recover for 15 days prior to experimentation.

Blood Sampling: Blood samples (500 µl) were obtained from the tail vein of conscious restrained rats at 0,1, 2, 4, 8, 16 and 24 hr following application of both testosterone (n=6) and testosterone phosphate (n=6) formulations. Blood was collected into EDTA tubes, then centrifuged at 5000 rpm for 10 min. Plasma was removed and stored at -80°C until assay.

Transdermal Formulation Preparation and Application: Testosterone and testosterone phosphate were provided by Tocovite Pty Ltd and prepared in the carrier from Example 1 approximately 1hr before application.

Testosterone Phosphate (***TP***): 4.41 mg of TP was dissolved in 15 ml of water and then made up to 100 ml with ethanol. 1 ml was transferred to an Eppendorf tube and the solvent was evaporated under a nitrogen stream. 1.00 g of the carrier from Example 1 was added and mixed with a glass rod and centrifuged. This was repeated 5 times.

Testosterone (***T***): 3.94 mg of T was dissolved in 15 ml of water and then made up to 100 ml with ethanol. 1 ml was transferred to an Eppendorf tube and the solvent was evaporated under a nitrogen stream. 1.00 g of the carrier from Example 1 was added and mixed with a glass rod and centrifuged. This was repeated 5 times.

Each formulation was applied to the dorsal skin of an anaesthetized rat in an area of approximately 4 cm^2 marked with an indelible felt tip marker. Application of approximately 30 mg of formulation (containing 1 µg of T or TP) was applied to the site with a curved glass rod applicator. The formulation was 'rubbed' in until it appeared to have been absorbed into the skin, which took between 5 to 10 min. Any changes in the consistency of the formulation during this procedure were noted.

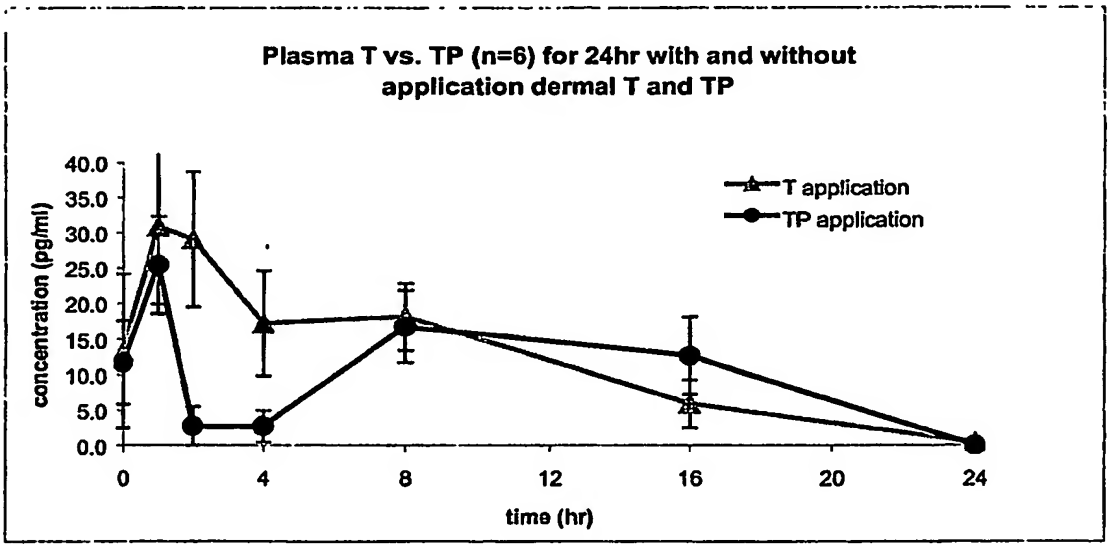
Results

Formulation Application: The average amounts of these formulations applied in the T and TP groups were 1 µg ± 0.02 µg.

Total Testosterone in Plasma: Measurable levels of testosterone (between standard curve range 2.5 – 100 pg/ml) were present in both groups of animals with maximum concentrations of 30.90 ± 11.00 (mean ± SD) pg/ml plasma measured in the T groups at 1, 8 and 16 hr post-application. Baseline measurements were taken at t=0 and these values were subtracted from all values to correct for background levels in the plasma.

Normal levels of testosterone for males is 437 to 707 pg/ml and in females is 24 to 47 pg/ml. The 1 µg dose applied in this study may therefore provide a therapeutic dose in females.

Figure 4: Changes in total testosterone (mean ± SD) measured in plasma samples obtained from ovariectomised hairless rats to which formulations containing approximately 1.00 µg ± 0.02 µg of T or TP were applied.



Discussion

The concentration of testosterone in blood increased when both testosterone and testosterone phosphate was applied. This suggests that testosterone and testosterone phosphate formulated in the carrier from example 1 provides an effective formulation for delivering testosterone.

The word 'comprising' and forms of the word 'comprising' as used in this description does not limit the invention claimed to exclude any variants or additions.

Modifications and improvements to the invention will be readily apparent to those skilled in the art. Such modifications and improvements are intended to be within the scope of this invention.

Simon Michael West and David Kannar **9 August 2002**